

Original Article

# Silymarine extract improved plasma homocysteine, lipids and liver enzymes in hyperhomocysteinemic non-alcoholic steatohepatitis

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## Abstract

**Introduction:** Silymarine extract is currently prescribed by some physicians for treatment of fatty liver disease. In the present study, the effect of administration of tablet containing silymarine extract on plasma homocysteine (Hcy), folate, vitamin B12 and liver enzymes activity in non-alcoholic steatohepatitis (NASH) was investigated.

**Materials and Methods:** Seventy-four patients (40 female and 34 male; aged 32-44 years) who were diagnosed with NASH and hyperhomocysteinemic by liver biopsy, measurement of liver enzymes activity and plasma Hcy were recruited for this study. The patients were not consuming alcohol or taking any other medications/medicines. Plasma Hcy, folate, B12, cholesterol, triglyceride, fasting glucose, aspartate aminotransferase (AST) and alaninaminotransferase (ALT) activity of all patients were measured before and after treatment. Tablet containing silymarine extract (140mg/kg) was administrated thrice /three times a day for 2 months.

**Results:** The results showed a significant reduction in the level of plasma Hcy, cholesterol, triglyceride, fasting glucose ( $P < 0.05$ ) following the administration of tablet containing silymarine extract, however, the increase in plasma folate and B12 levels was not significant. The results did not demonstrate any significant negative correlations between Hcy and B12, and also between Hcy and folate.

**Conclusion:** It can be concluded that consumption of tablet containing silymarine extract in NASH patients significantly reduces level of plasma Hcy, cholesterol, triglyceride, fasting glucose and AST and ALT activity, while level of plasma folate and B12 remained unchanged.

## Keywords:

Silymarin;  
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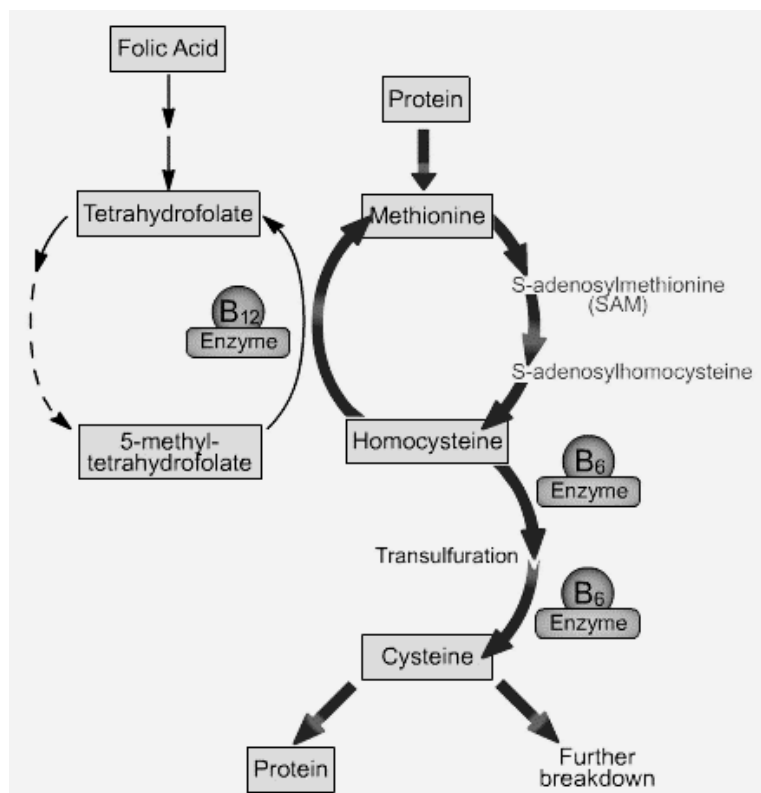
## Introduction

Non-alcoholic fatty liver disease (NAFLD) is one cause of fatty liver which occurs when fat is deposited

(steatosis) in the liver not due to excessive alcohol use. Non-alcoholic steatohepatitis (NASH) is the most extreme form of NAFLD, and is regarded as a major cause of cirrhosis of the liver of unknown cause. origin NAFLD appears to be strongly associated with

obesity, insulin resistance states including diabetes and other types of the metabolic syndroms, such as high triglycerides and low HDL. Most patients with NAFLD have few or no symptoms. Patients may complain of fatigue, malaise and dull right-upper-quadrant abdominal discomfort (Mehta et al, 2002, Marchesini et al, 2003, Luyckx et al., 2000). Homocysteine (Hcy) is a non-protein forming sulfur amino acid, synthesized from methionine (Met), whose metabolism is the result of two metabolic pathways (Fig.1): remethylation and trans-sulfuration. Plasma Hcy level is influenced by folate, vitamin B6, B12, methylene tetrahydrofolate reductase (MTHFR), cystathionine-b-synthase and s-adenosyl methionine (Refsum et al., 2004; Scott et al.,1998; Selhub et al ,1999). Folate is also an essential substrate in remethylation of homocysteine into methionine. In folate-dependent homocysteine remethylation, vitamin B12 (cobalamin) is a cofactor for methionine synthase. Vitamin B6 is a cofactor for cystathionine-b-synthase, which is necessary for the transsulfuration of homocysteine into cysteine (Selhub et al., 1999, Fowler et al, 2005; Finkelstein et al, 2007). Homocysteine was reported to be significantly higher

in the non-alcoholic steatohepatitis patients when compared with simple steatosis group, and Plasma homocysteine is a parameter for distinguishing steatohepatitis from simple steatosis (Gulsen et al, 2005). *Silybum marianum* L. (also known as: Milk thistle and *Carduus marianum*), a member of asteraceae family, is an ancient medicinal plant which has been used for centuries for treatment of different diseases such as liver and gallbladder disorders, protecting liver against snake bite /venom and insect stings, mushroom poisoning and alcohol abuse (Kren et al, 2005, Girish et al, 2006). Silymarin is the active component of this herb, which is a combination of other components, mainly silybin A, silybin, B, isosilybin A, isosilybin B and also other flavonolignants such as silychristin, neosilyhermin, silyhermin and silydianin which exist in its fruit and seeds (Dermarderosin et al, 2001, Kaur et al, 2007, Basiglio et al, 2009). Moreover, silymarin has antifibrotic, immunomodulating, anti-inflammatory (Dehmlow et al, 1996, Fantozzi et al, 1986) as well as antioxidant (Fathy et al,2009, Machlin, 1987) properties. It was also recommended for treatment of hepatitis, hepatic cirrhosis and mushroom poisoning



**Fig.1.** Homocysteine metabolism

(Cesanow et al, 2006, Karimi et al, 2005, WenWuet al, 2009). Silymarin extract in the form of tablet is currently prescribed in some of the clinics for treatment of fatty liver disease, while the exact mechanism of action of this drug on hepatic patients is not clear, one possibility is acting to improve Hcy level. Hence, this study was conducted to evaluate the effect of this herbal medicine on activity of liver enzymes, Hcy, vitamin B12 and plasma lipids in NASH patients.

## Materials and methods

### Study design

Seventy-four patients (40 women and 34 men; aged 32-44 years) who were diagnosed as hyperhomocysteinemic and NASH following measurement of plasma homocystein, liver enzymes and liver biopsy were assigned/recruited for this study. The patients were not consuming alcohol nor taking any other medications/medicines. All patients were negative for the disease markers such as hemochromatosis, autoimmune, Willson and viral hepatitis. In the selected patients, 24 were obese (BMI>30), 11 had decrease in folate, 9 had decrease of B12 levels, 27 patients showed mild hyperglycemia and 23 patients were hyperlipidemic (increase in total cholesterol and triglyceride). All patients had elevated ALT and total Hcy. Tablet (Goldaru, Iran) containing silymarin extract (140mg/kg) was administered to all patients three times a day for 2 months.

### Analytic procedures in serum samples:

Fasting venous blood samples were collected from all patients in the beginning and in the final day of treatment, between 08:00 and 08:30 a.m. after 12 h fasting and centrifuged at 3000 × g for 10 min within <60 min, which is sufficient to prevent an increase in plasma Hcy resulting from *ex vivo* generation of Hcy by erythrocytes. Plasma was stored at -80 °C Hcy was measured.

Total homocysteine (tHcy) concentration which refers to the sum of protein-bound, free-oxidized,(radicals) and reduced species of homocysteine in plasma was

determined by the Axis® Homocysteine EIA kit (Alirezaei et al, 2011, 2012). The samples were prepared according to manufacturer's instructions. Absorbance was measured at a wavelength of 450 nm using an ELISA reader (STAT FAX 2100, USA). All estimations were performed in duplicate and the intra-assay coefficient of variation was <10 % and the detection limit of the tHcy assay was 2.0 µM. The tHcy results were expressed as micromoles per liter of plasma (µmol/l).

Serum aspartate transaminase (AST), alanine transaminase (ALT) activity, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and glucose were measured within 1 h after blood was drawn, with standard methods using an automated analyser (Hitachi 911, Germany). Sera were also immediately frozen at -20 °C for measurement of folate and vitamin B12.

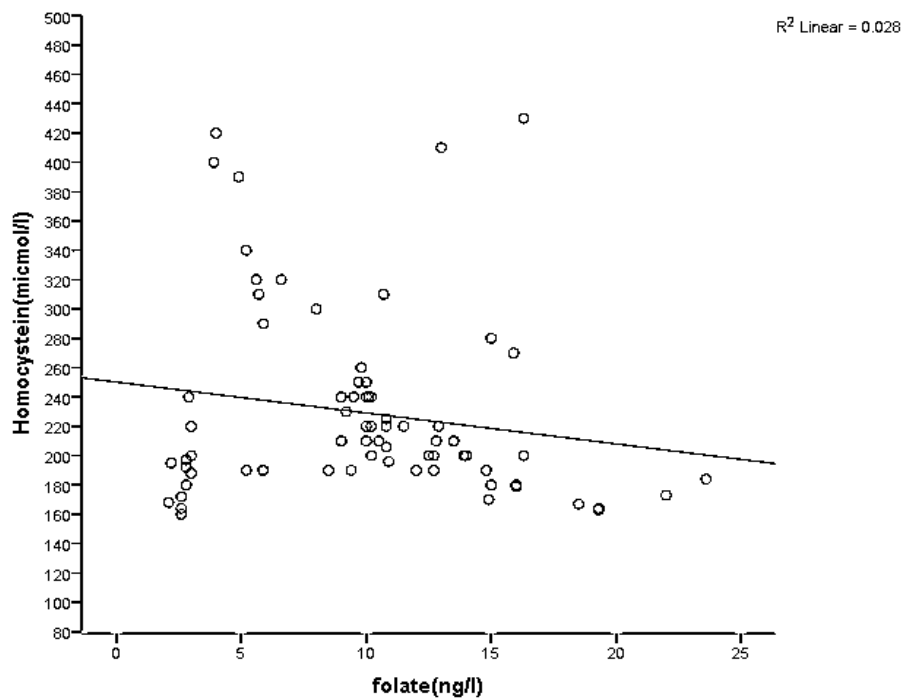
Vitamin B12 and folate were measured with immunochemiluminescence by COBAS system (Germany). The detection limit for folate and cobalamin was 1.36 nmol/L and 22 pmol/L respectively.

### Statistical analysis

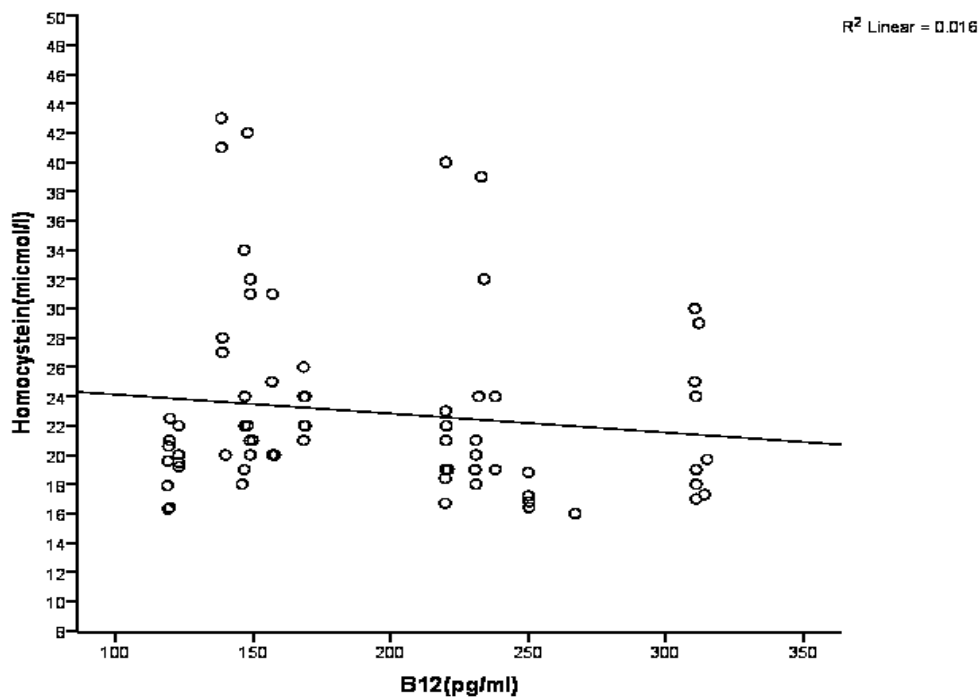
Data obtained from men and women were compared by independent-t-test. Comparison of data between beginning and end of treatment period was done, using paired-t-test. Pearson's coefficient was used to discover a correlation between Hcy and B12, also Hcy and folate. The quantitative variables were described by mean±SEM. In statistical analysis, p<0.05 was taken as significant.

## Results

The results didn't demonstrate any significant differences between male and females, hence all patients were considered as single group and their data were compared before and after treatment. The results showed a significant reduction in the level of plasma Hcy (Table1), cholesterol, triglyceride, fasting glucose (Table 2) and activity of AST and ALT following consumption of silymarin extract (P <0.05) (Table3). Level of plasma folate and B12 did not change significantly (Table1). Moreover, the results did not demonstrate a significant negative correlation



**Fig. 2.** Correlation between plasma Hcy and folate No significant correlation was observed between parameters ( $P > 0.05$ )



**Fig. 3.** Correlation between plasma Hcy and B12 No significant correlation was observed between parameters ( $P > 0.05$ )

between Hcy and folate ( $P > 0.05$ ) (Fig.2) and also between Hcy and vitamin B12 ( $P > 0.05$ ) (Fig.3).

## Discussion

Our results showed significant reduction in the level of

serum Hcy, cholesterol, triglyceride, fasting glucose and liver enzymes following consumption of tablet containing silymarine extract. To the best of our knowledge this is the first report about the effect of tablet containing silymarine extract on tHcy, B12 and folate. These findings about tHcy support the reports by Carvalho et al (2013) and Gulsen et al (2005)

**Table 1:** Comparison between (meant $\pm$  SEM) plasma tHcy, folate and B12 levels before and after treatment with silymarine extract

parameters	Total Hcy ( $\mu$ mol/L)	Folate (Pg/ml)	B12 (ng/ml)
Time of sampling			
Before treatment	22.140 $\pm$ 0.423	106.200 $\pm$ 2.029	2.408 $\pm$ 0.080
After treatment	* 16.280 $\pm$ 0.350	107.900 $\pm$ 1.920	2.508 $\pm$ 0.800

\* indicate P&lt;0.05

**Table 2:** Comparison between (mean  $\pm$ SEM) plasma triglyceride, cholesterol and glucose fasting levels before and after treatment with silymarine extract

parameters	Triglycerides (mg/d1)	Cholesterol (mg/d1)	FBS (mg/d1)
Time of sampling			
Before treatment	232.750 $\pm$ 5.591	242.880 $\pm$ 1.893	141.330 $\pm$ 1.575
After treatment	*214.625 $\pm$ 4.217	*216.880 $\pm$ 1.505	*119.930 $\pm$ 1.575

\* indicate P&lt;0.05

**Table 3:** Comparison between (mean  $\pm$ SEM) serum AST and ALT levels before and after treatment with silymarine extract

parameters	ALT (IU/l)	AST (IU/l)
Time of sampling		
Before treatment	52.120 $\pm$ 0.751	48.730 $\pm$ 0.825
After treatment	*41.000 $\pm$ 0.520	*40.270 $\pm$ 0.463

\* indicate P&lt;0.05

regarding high level of plasma tHcy in non-alcoholic fatty liver patients. Reduction of fasting glucose, cholesterol, triglyceride, AST and ALT in type 2 diabetes mellitus was also reported following consumption of silymarin (Husaini et al 2006). It was also reported to recover endocrine function and affect morphology in diabetic models (Soto et al, 2010). Hepatoprotective properties of silymarin may be due

to free radical scavenging and raising the cellular content of glutathione that lead to the lipid peroxidation inhibition, increasing membrane stability in exposure to xenobiotics, steroid-like effect via adjustment of nuclear expression and reducing the deposition of collagen fibers, as silymarin inhibits the conversion of stellate hepatocytes into myofibroblasts. In addition, silymarin/silybin increases ribosomal

protein synthesis by means of stimulating RNA polymerase I (Saller et al, 2007). Silymarin increases serum insulin, reduces serum glucose and increases antioxidant enzymes and glutathione. Moreover, silybin has a chemoprotective effect and can improve pancreatic function after exposure to toxic agents leading to damages (Soto et al, 2004, Soto et al, 2002, Soto et al, 2003). It is likely that the mechanism of action of silymarin is through acting on kinetics of glucose-6 phosphatase and inhibition of gluconeogenesis, reduced 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA) (Bok et al., 1999; Davalos et al., 2006).

Our results did not demonstrate significant increase of plasma folate and B12 levels. Also, no significant negative correlation was observed between level of Hcy and B12, and between level of Hcy and folate. Some controversy exists regarding the association between vitamin B12 or folate and NAFLD. Some authors have reported similar level (Hirsch et al. 2005, Polyzos et al, 2012), whereas others lower (Gulsen et al. 2005, Koplay et al. 2011) or even higher (Sazci et al. 2008) vitamin B12 and/or folate levels in NAFLD patients than controls. Gulsen et al. (2005) reported a significant negative correlation between Hcy and folate and also between hcy and B12. Carvalho et al. (2013) reported negative correlation between levels of vitamin B12 and Hcy concentration in NAFLD too. These differences could be partly attributed to the potential influence of multiple factors on serum folate and vitamin B12 levels, including age, body weight, gender, menopause and ethnicity, as well as assay conditions (Weggemans et al. 1997; Carmel et al. 1999; Pfeiffer et al. 2011).

## Conclusion

Consumption of silymarin extract by NASH patients significantly reduced level of plasma Hcy, cholesterol, triglyceride, fasting glucose, AST and ALT, while level of plasma folate and B12 did not change significantly.

## Conflict of interest

Authors have no declarations of interest to report.

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